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FILE 'USPAT' ENTERED AT 10:38:03 ON 19 APR 1999

WELCOME TO THE U.S. PATENT TEXT FILE

L1 1399 S PERTUSSIS
L2 5194 S TOXIN OR HOLOTOXIN
L3 331 S L1(5N)L2
L4 870243 S MUTAT? OR SUBSTITUT? OR MUTANT? OR MUTAGEN? OR REPLACE?
L5 21977 S ARG OR ARGININE
L6 1990 S L5(5N)L4
L7 7 S L6(P)L3
L8 11 S L6 AND L3 NOT L7
L9 3 S L1(P)L5(P)L4 NOT L7
L10 16 S L1(P)L5 NOT (L7 OR L9 *

L8

1. 5,889,172, Mar. 30, 1999, DNA sequences for immunologically active peptides of **pertussis** **toxin**; Mariagrazia Pizza, et al., 536/23.7; 424/190.1, 254.1, 832; 435/69.3 [IMAGE AVAILABLE]

2. 5,874,287, Feb. 23, 1999, Mutagenized DNA molecules encoding modified subunit a of Cholera-toxin; W. Neal Burnette, et al., 435/252.3, 252.33, 254.1, 325, 909; 536/23.2 [IMAGE AVAILABLE]

3. 5,856,122, Jan. 5, 1999, Modification of **pertussis** **toxin**; Randy J. Read, et al., 435/69.1, 15 [IMAGE AVAILABLE]

4. 5,834,310, Nov. 10, 1998, Mammalian muscle NAD: arginine ADP-ribosyltransferase; Joel Moss, et al., 435/325, 193, 194, 252.3, 252.33, 320.1, 350, 351, 352, 353, 354; 536/23.1, 23.2, 23.5 [IMAGE AVAILABLE]

US PAT NO: 5,856,122 [IMAGE AVAILABLE] L8: 3 of 11
TITLE: Modification of **pertussis** **toxin**

ABSTRACT: The three-dimensional structure of crystalline **pertussis** **holotoxin** (PT) has been determined by X-ray crystallography. Crystal structures have also been determined for complexes of **pertussis** **toxin** with molecules relevant to the biological activity of PT. These three-dimensional structures were analyzed to identify functional amino acids appropriate. . . be used to predict amino acids which contribute to the toxicity of the holotoxin, to produce immunoprotective, genetically-detoxified analogs of **pertussis** **toxin**.

BSUM(2 The present invention relates to a method for the prediction of functional amino acid residues in **pertussis** **toxin**, in order to manipulate the biological properties of the toxin, by determination of and examination of the crystal structures of. . .

BSUM(4 Whooping . . . of the disease is still not fully understood; however, it is generally recognized that major systemic effects are caused by **pertussis** **toxin** (PT). This material exhibits a wide range of biological activities, as illustrated by such alternative names as lymphocytosis-promoting factor, histamine-sensitizing. . .

BSUM(9) The . . . toxin (LT) (refs. 16,17). Amino acids in this region that contribute to the ADP-ribosyltransferase activity of PT were identified by **substitution** **mutagenesis**. In particular, the **Arg**-9 to Lys-9 **replacement** was found to greatly reduce enzymatic activity (ref. 18). A second region of S1, located between Val-51 and Tyr-59, is.

. .

BSUM(10) **Pertussis** **toxin** has also been detoxified by modification of itscell binding properties, for example by deletion of Asn-105 in the S2. ..

BSUM(16) In . . . the present invention, there is provided a method of predicting at least one site contributing to the biological activity of **pertussis** **holotoxin**, which comprises analyzing a three-dimensional structure of crystalline **pertussis** **holotoxin** determined by X-ray crystallography in relation to known information concerning protein structure and function to identify the at least one. . .

BSUM(17) Such a biological activity of **pertussis** **holotoxin** may include toxicity, cell-binding, mitogenicity, enzymatic activity and adjuvanticity of the **pertussis** **holotoxin**. The at least one site which is predicted by the method provided herein may comprise a single amino acid or. . .

BSUM(18) Such analyzing step may comprise comparing the three-dimensional structure of **pertussis** **holotoxin** with known three-dimensional structures of enzymes with substantial functional resemblance to **pertussis** **holotoxin** (including bacterial toxins having ADP-ribosyl transferase activity), and identifying structurally conserved regions between the **pertussis** **holotoxin** and the enzymes.

BSUM(19) Such analyzing step also may comprise comparing the three-dimensional structure of **pertussis** **holotoxin** with known three-dimensional structures of other proteins with carbohydrate binding properties, and identifying regions of structural resemblance of the **pertussis** **holotoxin** to the proteins.

BSUM(20) I n these procedures, analysis may also be effected by aligning amino acid sequences of **pertussis** **holotoxi with those of the enzymes or proteins with carbohydrate binding properties, as the case may be, according to structural equivalence. . .

BSUM(21) The analyzing step further may comprise locating, within the three-dimensional structure of **pertussis** **holotoxin**, amino acid residues known to contribute to the biological activity of the holotoxin, and identifying spatially-proximate amino acid residues interacting. ..

BSUM(22) Following . . . the procedure provided herein, the identified at least one site may be modified to alter the biological activity of the **pertussis** **holotoxin**, which modification may be effected by genetic, chemical or biochemical means.

BSUM(23) Accordingly, the present invention includes the use of the three-dimensional structure of crystalline **pertussis** **holotoxin** determined by X-ray crystallography for identifying at least one site in the **pertussis** **holotoxin** molecule contributing to the biological activity, including any of the activities noted above.

BSUM(24) In . . . with a further aspect of the present invention, there is provided a method of identifying at least one site in **pertussis** **holotoxin** that interacts with a molecule that is capable of forming a complex with the holotoxin, the method comprising:

BSUM(25 (a) providing a crystalline complex between at least a portion of**pertussis** **holotoxin** and the molecule;

BSUM(28)The at least one identified site may contribute to toxicity, cell binding, mitogenicity, enzymatic activity or adjuvanticity the **pertussis** **holotoxin**.

BSUM(29) The at least a portion of the holotoxin with which the complex is formed may be the entire **pertussis** **holotoxin**, an analog thereof, a subunit of the holotoxin, a portion of a subunit, or a combination of subunits.

BSUM(31) The . . . for the enzymatic activity of PT. Such substrates include NAD and analogs of NAD that are not substantially hydrolysable by **pertussis** **toxin**. The substrate may also be a GTP-binding protein (a G-protein), an .alpha.-subunit of a GTP-binding protein or a C-terminal fragment. . .

BSUM(32 Following . . . biochemical, or chemical means, to alter a biological activity, such as toxicity, enzymatic activity, mitogenicity cell-binding and adjuvanticity, of the **pertussis** **holotoxin**.

BSUM(34 The present invention further comprises a crystalline form of isolated **pertussis** **holotoxin** in which the molecules of **pertussis** **toxin** have the three-dimensional structure represented by FIGS. 1 and 2 described below. The crystalline form of **pertussis** **holotoxin** may be in dimeric form, as shown in FIG. 8, described below. The crystalline form of **pertussis** **holotoxin** may have a space group P2.sub.1 2.sub.1 2.sub.1 with cell dimensions a=163.8 .ANG., b=98.2 .ANG. and c=194.5 .ANG.. The crystalline form of **pertussis** **holotoxin** may be in the form of a complex with a molecule capable of forming a complex with the holotoxin, as. . .

BSUM(35 In addition, the present invention includes a crystalline form of isolated **pertussis** **holotoxin** characterized by atomic co-ordinates specified in accession no. 1 PRT of the Brookhaven Protein Data Bank, Brookhaven, N.Y. USA.

BSUM(36 The provision of a crystalline form of **pertussis** **holotoxin** allows a comparison with other proteins having functional resemblance to pertussis holotixin (for example bacterial toxins from Campylobacter jejuni and. . . identify currently unknown sites that contribute to toxicity of such toxins by a comparison with the three dimensional structure of **pertussis** **holotoxin** provides a technique for detoxification of such toxins to provide useful immunogenic but non-toxic analogues.

BSUM(37 The crystalline form of **pertussis** **holotoxin** as provided hereinis of a particularly high purity and is useful as a primary standard formeasuring the quantity,. . .

BSUM(38 The present invention further includes a method for the production of amodified **pertussis** **holotoxin**, which comprises (a) identifying atleast one amino acid residue of the holotoxin for modification byutilizing the prediction procedure. . .

BSUM(39 In . . . aspect of the invention, there is provided a method for producing a modified form of at least a portion of **pertussis** **holotoxin**, comprising (a) forming a crystalline complex between at least a portion of **pertussis** **holotoxin** and a molecule capable of complexing with the holotoxin; (b) determining a three-dimensional structure of the complex; (c) analysing the structure to identify at least one amino acid residue of the at least a portion of **pertussis** **holotoxin** interacting with the molecule; (d) effecting mutagenesis of a nucleotide sequence encoding the at least a portion of the **pertussis** **holotoxin** to remove or replace a codon for the at least one amino acid and/or to insert at least one codon. . . nucleotide sequence; and (e) expressing the mutant nucleotide sequence to produce the modified form of at least a portion of **pertussis** **holotoxin**. The at least a portion of **pertussis** **holotoxin** may be **pertussis** **holotoxin** or an analog thereof and the step of expressing the mutant nucleotide sequence is effected in a Bordetella organism. Alternatively, the at least a portion of **pertussis** **holotoxin** may be a subunit of the holotoxin, a portion of such a subunit or a combination of subunits. The at. . .

BSUM(42 The invention additionally includes a mutant **pertussis** **holotoxin** wherein at least one amino acid residue in the S1, S2, S3, S4 or S5 subunits is substituted by another. . .

BSUM(44 The mutant **pertussis** **holotoxin** may comprise modification of at least one amino acid residue located from 184 to 203 or located from 211 to. . .

BSUM(45 The mutant **pertussis** **holotoxin** provided herein may further comprise at least one additional amino acid provided at the C-terminal end of the S1 subunit,. . .

DRWD(3 FIG. 1 shows a schematic representation of the B-oligomer of **pertussis** **toxin** viewed along the pseudo-5-fold axis from the side opposite to S1. Subunit S2 is shown in pale blue, S3 in. . .

DRWD(4 FIG. 2 shows a schematic representation of **pertussis** **toxin** viewed perpendicular to the 5-fold axis of the B-oligomer. Subunit S1 is shown in green, and the other subunits as. . .

DRWD(5 FIG. 3 shows a schematic representation of the active site of subunit S1 of **pertussis** **toxin** with individual amino acid residues identified according to the standard one-letter coding system;

DETD(4 This Example describes the crystallization of **pertussis** **toxin** (PT), data collection and phase determination.

DETD(10 The atomic co-ordinates of the crystalline **pertussis** **holotoxin** as determined herein have been deposited as Accession Number 1 PRT of the Brookhaven Protein Data Bank, Brookhaven, N.Y., USA.

DETD(12 This Example describes model building of the **pertussis** **toxin** molecule.

DETD(18 This Example shows the analysis of the structure of **pertussis** **toxin**.

DETD(36 This Example describes the identification of functional amino acid residues in **pertussis** **toxin** by examination of its 3D structure.

DETD(41) The . . . is occupied by amino acid residues His-15, Gln-16, Leu-82 and Lys-83 in S2, and by residues Gln-15, Gln-16, Tyr-82 and **Arg**-83 in S3. **Replacement** of Tyr-82 of S3 has already been shown to diminish the biological activity of PT (ref. 22), but the significance. . .

DETD(64 This Example describes the analysis of the structure of the complex between **pertussis** **toxin** and the undecasaccharide from human serum transferrin, and the identification of functional amino acid residues in **pertussis** **toxin**.

DETD(76 This Example describes the analysis of the structure of the complex between **pertussis** **toxin** and ATP, and the identification of functionally important amino acid residues in **pertussis** **toxin**.

DETD(80 The . . . to the overall binding energy of ATP. This conclusion is consistent with studies that show that binding of ADP to **pertussis** **toxin** is considerably weaker than that of ATP (refs. 3,4).

DETD(84 The functional importance of ATP binding to **pertussis** **toxin** lies in its ability to loosen the interaction between the catalytic Si subunit and the B-oligomer, or allow its complete. . .

DETD(105 The present invention also provides **pertussis** **toxin** analogues in which at least one of the amino acid residues from position 211 to position 220 of subunit S1. . .

DETD(109 The present invention further provides **pertussis** **toxin** analogues in which the hydrophobic region between residues 184 and 203 of subunit S1 is rendered more hydrophilic by substitution. . .

DETD(110 Referring to Table 6, there is shown the characterization of PT analogs <——User Break——>
The . . . (a) identifying at least one amino acid residue of the holotoxin for modification by analyzing a three-dimensional structure of crystalline **pertussis** **holotoxin** determined by X-ray crystallography in relation to known information concerning protein structure and function; (b) effecting mutagenesis of a tox. . .

CLMS(1 What we claim is:
1. A method of preparing a **pertussis** **holotoxin** having a modified biological activity, which comprises: (A) identifying at least one site in a **pertussis** **holotoxin** that interacts with the molecule that is capable of forming a complex with the holotoxin and which molecule is an effector molecule which is an adenine nucleotide and which site contributes to toxicity, cell binding or enzymatic activity of **pertussis** **holotoxin** by
(a) forming a crystalline complex between at least a portion of **pertussis** **holotoxin** which is **pertussis** **holotoxin**, an analog thereof, a subunit, a portion of a subunit or a combination of subunits and said molecule, including exposing crystals of the at least a portion of the holotoxin to the molecule, under conditions to effect formation of the crystalline complex without substantial disruption of the crystals;
(b) determining the three-dimensional structure of the complex by X-ray crystallography;
(c) analyzing the structure to identify the at least one interacting site; and
(B) modifying said identified at least one site to alter the toxicity, cell binding or enzyme activity of said pertassis holotoxin.

2. The method of claim 1, wherein the adenine nucleotide is ATP.

3. A method of preparing a **pertussis** **holotoxin** having a modified biological activity, which comprises:
(A) identifying at least one site in **pertussis** **holotoxin** that interacts with a molecule that is capable of forming a complex with the holotoxin and which molecule is a substrate which is a GTP-binding protein, an .alpha.-subunit of a GTP-binding protein, or a (C-terminal fragment of an .alpha.-subunit of a GTP-binding protein and which site contributes to toxicity, cell binding or enzymatic activity of **pertussis** **holotoxin** by:
(a) forming a crystalline complex between at least a portion of **pertussis** **holotoxin** which is **pertussis** **holotoxin**, an analog thereof, a subunit, a position of a subunit or a combination of subunits and said molecule, including exposing crystals of the at least a portion of the holotoxin to the molecule, under conditions to effect formation of the crystalline complex without substantial disruption of the crystals;
(b) determining the three-dimensional structure of the complex by X-ray crystallography; and
(c) analysing the structure to identify the at least one interacting site; and
(B) modifying said identified at least one site to alter the toxicity, cell binding or enzymatic activity of said **pertussis** **holotoxin**.

4. The method of claim 3, wherein the GTP-binding protein is selected from the group consisting of G.sub.i, G.sub.o, and transducin.

5. A method of preparing a **pertussis** **holotoxin** having a modified biological activity, which comprises:
(A) identifying at least one site in **pertussis** **holotoxin** that interacts with molecule that is capable of forming a complex with the holotoxin and which molecule is a substrate which is NM or a substantially non-hydrolysable analog of NAD and which site contributes to toxicity, cell binding or enzymatic activity of **pertussis** **holotoxin** by:
(a) forming a crystalline complex between at least a portion of **pertussis** **holotoxin** which is **pertussis** **holotoxin**, an analog thereof, a subunit, a portion of a subunit or a combination of subunits and said molecule, including exposing crystals of the at least a portion or the holotoxin to the molecule, under conditions to effect formation of the crystalline complex without substantial disruption of the crystals;
(b) determining the three-dimensional structure of the complex by X-ray crystallography;
(c) analyzing the structure to identify the at least one interacting site; and
(B) modifying said identified at least one site to alter the toxicity, cell binding or enzymatic activity of said **pertussis** **holotoxin**.

1. 5,889,172, Mar. 30, 1999, DNA sequences for immunologically active peptides of pertussis toxin; Mariagrazia Pizza, et al., 536/23.7; 424/190.1, 254.1, 832; 435/69.3 [IMAGE AVAILABLE]

2. 5,856,122, Jan. 5, 1999, Modification of pertussis toxin; Randy J. Read, et al., 435/69.1, 15 [IMAGE AVAILABLE]

US PAT NO: 5,889,172 [IMAGE AVAILABLE] L9: 1 of 3
CLAIMS: We claim:
1. An isolated DNA sequence coding for the S1 subunit of the **pertussis** toxin gene wherein the bases coding for glutamic acid at amino acid position 129 are **replaced** by the bases coding for glycine, and the bases coding for **arginine** at amino acid position 9 are **replaced** by the bases coding for another amino acid.

2. The isolated DNA sequence of claim 1 wherein the bases coding for arginine at amino acid position 9 are replaced by the bases coding for the amino acid glycine.

L10
1. 5,877,298, Mar. 2, 1999, Acellular pertussis vaccines and methods of preparing thereof; Raafat E. F. Fahim, et al., 530/412, 413, 414, 415, 417, 418, 419, 421, 422 [IMAGE AVAILABLE]

2. 5,840,674, Nov. 24, 1998, Covalent microparticle-drug conjugates for biological targeting; Milton B. Yatvin, et al., 510/392; 435/188; 510/465, 530 [IMAGE AVAILABLE]

3. 5,834,310, Nov. 10, 1998, Mammalian muscle NAD: arginine ADP-ribosyltransferase; Joel Moss, et al., 435/325, 193, 194, 252.3, 252.33, 320.1, 350, 351, 352, 353, 354; 536/23.1, 23.2, 23.5 [IMAGE AVAILABLE]

US PAT NO: 5,877,298 [IMAGE AVAILABLE] L10: 1 of 16
DETD(267) 36. . . . E., Roberts, M., Kenimer, J. G., Charles, I. G., Fairweather, M., Novotny, P., and Brennan, M. J (1991). Pertactin, and **Arg**-Gly-Asp-containing Bordetella **pertussis** surface protein that promotes adherence of mammalian cells. Proc. Natl. Acad Sci. USA 88: 345-349.

CLAIMS: What we claim is:
1. A process for preparing an agglutinogen preparation comprising fimbrial agglutinogens 2 (Agg 2) and fimbrial agglutinogen 3 (Agg 3) free from agglutinogen 1 from a Bordetella strain, comprising the steps of:
(a) providing a cell paste of the Bordetella strain;
(b) selectively extracting fimbrial agglutinogens 2 and 3 from the cell paste by dispersing the cell paste in a buffer comprising about 1M to about 6M urea to produce a first supernatant containing said agglutinogens 2 and 3 and a first residual precipitate;
(c) separating the first supernatant from the first residual precipitate;
(d) incubating the first supernatant at a temperature of about75.degree. C. to about 85.degree. C. and for a time of about 10 minutes to about 60 minutes to produce a clarified supernatant containing fimbrial agglutinogens 2 and 3 and a second precipitate containing non-fimbrial agglutinogen contaminants;
(e) concentrating the clarified supernatant to produce a crude fimbrial agglutinogen solution by precipitating fimbrial agglutinogens 2 and 3 from the clarified supernatant by the addition of a polyethylene glycol to the clarified supernatant, separating the precipitated fimbrial agglutinogen 2 and 3 from the resulting supernatant and solubilizing the separated fimbrial agglutinogens 2 and 3; and
(f) purifying fimbrial agglutinogens 2 and 3 from the crude fimbrial agglutinogen solution to produce the fimbrial agglutinogen preparation comprising fimbrial agglutinogens 2 and 3.

2. The process of claim 1 wherein the temperature is about 80.degree. C.

3. The process of claim 1 wherein the time is about 30 minutes.

4. The process of claim 1 wherein the first supernatant is concentrated prior to the incubation step (d).

5. The process of claim 1 wherein said precipitation is effected by adding polyethylene glycol of molecular weight about 8000 to the clarified supernatant to a concentration of about 3% to about 5 wt. % to effect precipitation of said agglutinogens from the clarified supernatant.

6. The process of claim 5 wherein the concentration of polyethylene glycol is about 4.3 to about 4.7wt%.

7. The process of claim 1 wherein the agglutinogens are purified from the crude fimbrial agglutinogen solution by column chromatography.

8. The process of claim 7 wherein said column chromatography includes SEPHADEX 6B and/or PEI silica column chromatography.

9. The process of claim 7 wherein said purification step includes sterilization of run through from said column chromatography purification to provide a sterile fimbrial agglutinogen preparation.

10. The process of claim 9 wherein said sterile fimbrial agglutinogen preparation is absorbed onto a mineral salt adjuvant.

11. The process of claim 10 wherein said mineral salt adjuvant is alum.

12. The process of claim 1 wherein the Bordetella strain is a strain of Bordetella pertussis.

US PAT NO: 5,840,674 [IMAGE AVAILABLE] L10: 2 of 16
DETD(12 Examples . . . are not limited to a urea-based linker for use against a pathogen which produces urease (e.g., Mycobacteria spp. and B. **pertussis**); a peptide linker comprised of (AlaAlaAlaAla).sub.n, wherein n can be an integer from 1-5, for use against a pathogen that. . . against human immunodeficiency virus 1 producing a specific protease termed HIV-1 protease; a peptide comprising the amino acid sequence: --Ala--Xaa--Cys.sub.Acm --Tyr--Cys--**Arg**--Ile--Pro--Ala--Cys.sub.Acm --Ile--Ala--Gly--Asp--**Arg**--**Arg**--Tyr--Gly--Thr--Cys.sub.Acm --Ile--Tyr--Gln--Gly--**Arg**--Leu--Trp--Ala--Phe--Cys.sub.Acm --Cys.sub.Acm --, wherein the microbial pathogen expresses an enzymatic activity that specifically disables the endogenous antimicrobial peptide defensin (e.g., Mycobacterium. . .

US PAT NO: 5,834,310 [IMAGE AVAILABLE] L10: 3 of 16
BSUM(8 Several . . . have similarities to one another in their amino acid sequences, the enzymes differ in the amino acids that they modify. **Arginine**, cysteine, asparagine and diphthamide (modified histidine) serve as ADP-ribose acceptors for Cholera toxin, **pertussis** toxin, botulinum C3 transferase and diphtheria toxin respectively.

Welcome to DIALOG
19apr99 11:15:40 User208600 Session D1189.1
File 357:Derwent Biotechnology Abs 1982-1999/Apr B2 (c) 1999 Derwent Publ Ltd

Set	Items	Description
S1	478	PERTUSSIS
S2	1156	ARGININE
S3	2	S1 AND S2
S4	7016	SUBSTITUT?
S5	32	S1 AND S4

Ref	Items	Index-term
E1	6	AU=BURNETT W V
E2	1	AU=BURNETT-CURLEY D
E3	0	*AU=BURNETTE
E4	1	AU=BURNETTE III W N
E5	15	AU=BURNETTE W N
E6	1	AU=BURNHAM C E
E7	1	AU=BURNHAM III W V
E8	3	AU=BURNHAM M
E9	2	AU=BURNHAM M K
E10	26	AU=BURNHAM M K R
E11	1	AU=BURNHAM M K R; PRATT J M; ROSENBERG M; WARD J
E12	2	AU=BURNIE J

S6	16	E4-E5
S7	4	S5 AND S6

3/7/1 DIALOG(R)File 357:Derwent Biotechnology Abs (c) 1999 Derwent Publ Ltd. All rts. reserv.
0127026 DBA Accession No.: 91-14668
Site-specific mutagenesis of the catalytic subunit of cholera toxin: substituting lysine for arginine 7 causes loss of activity - potential application as recombinant vaccine component
AUTHOR: Burnette W N; Mar V L; Platler B W; Schlotterbeck J D; McGinley M D; Stoney K S

CORPORATE AFFILIATE: Amgen CORPORATE SOURCE: Amgen Inc., Thousand Oaks, California 91320, USA.
JOURNAL: Infect.Immun. (59, 11, 4266-70) 1991 CODEN: INFIBR LANGUAGE: English
ABSTRACT: Genes encoding the cholera toxin (CTX) catalytic A subunit (CTXA) and the cholera toxin homopentameric B oligomer (CTXB) were individually subcloned into plasmid pUC19 vectors. Using appropriate synthetic oligonucleotide linkers, the genes for CTXB, CTXA (CTXA1 (N-terminal protein) + A2 (C-terminal protein)), CTXA1, and both CTXA and CTXA1 with the Arg-7 codon substituted by that for Lys, were subcloned into plasmid pUC19 vectors. After cultivation at 37 deg, induction of gene expression at 42 deg and cell disintegration, recombinant CTX subunits were recovered as insoluble refractile bodies and characterized. The effect of substituting Lys for Arg-7 in both CTXA and CTXA1 was identical and unambiguous when evaluated under 3 sets of assay conditions i.e. complete abolition of detectable ADP-ribosyltransferase activity. The striking loss of enzymatic activity was similar to that observed for a pertussis toxin (PTX) S1 analog with Lys-9 instead of Arg-9, and indicated that the CTXA Arg-7 and PTX S1 Arg-9 residues share functional homology. The recombinant CTXA may be useful as a recombinant vaccine component. (33 ref

3/7/2 DIALOG(R)File 357:Derwent Biotechnology Abs (c) 1999 Derwent Publ Ltd. All rts. reserv.
0123957 DBA Accession No.: 91-11599
Genetically inactivated pertussis toxins: vaccine candidates and virulence of toxoid producing Bordetella pertussis strains - pertussis toxin operon cloning (conference abstract)
AUTHOR: Brown D R; Keith J M; Sato H; Sato Y
CORPORATE SOURCE: NIDR, National Institute of Health, Bethesda, MD 20892, USA.
JOURNAL: Abstr.Gen.Meet.Am.Soc.Microbiol. (91 Meet., 72) 1991 CODEN: 0005P LANGUAGE: English
ABSTRACT: Cloning and sequencing of the pertussis toxin operon permitted the introduction of specific mutations in the S1 gene which had profound effects on the enzyme activity. Arginine at position 9 was converted to lysine, and doubly mutated S1 genes were constructed by addition of 4 changes of glutamic acid at position 129. The pertussis toxin operons were reassembled and inserted into the Bordetella pertussis chromosome. Toxoids were assembled and expressed to approximately wild type levels. ADP-ribosylation of purified transducin, lymphocytosis promotion and histamine sensitization activities were greatly reduced. Mice were immunized with the toxoids and the antisera was used in challenge assays. Reduced virulence was found in intracerebral and aerosol challenge assays by these toxoid-producing B. pertussis strains. (0 ref)

5/6/1 0232647 DBA Accession No.: 99-02748
Detoxified mutants of bacterial ADP-ribosylating toxins as parental adjuvants - e.g. Bordetella pertussis mutant pertussis toxin, Escherichia coli mutant heat-labile toxin with virus, tumor, etc. antigen or self-antigen, used as a vaccine for e.g. AIDS, cancer, etc. 1998

5/6/2 0219817 DBA Accession No.: 98-01414
Co-expression of LTK63 and DTP antigens in S. typhi vaccine strain CVD 980htrA - Escherichia coli heat-labile enterotoxin mutant co-expression in Salmonella typhi for use as a recombinant vaccine (conference abstract) 1997

5/6/3 0212400 DBA Accession No.: 97-07521
New microorganisms which produce RTX toxin - mutant gene cloning and expression in e.g. Pasteurella haemolytica, for use as a recombinant vaccine 1997

5/6/4 0212399 DBA Accession No.: 97-07520
New microorganisms which produce inactivated leukotoxin - mutant gene cloning and expression in e.g. Pasteurella haemolytica, for use as a recombinant vaccine 1997

5/6/5 0212385 DBA Accession No.: 97-07506
Rational design of vaccines - pertussis toxin protein engineering for detoxification, and expression in Bordetella pertussis for use as a recombinant vaccine 1997

5/6/6 0207414 DBA Accession No.: 97-02535
Expression and secretion of the S2 subunit of pertussis toxin in Bacillus brevis - Bordetella pertussis toxin recombinant protein secretion for use as a recombinant vaccine 1996

5/6/7 0182075 DBA Accession No.: 95-06963
New modified forms of pertussis holotoxin - recombinant pertussis toxin production by site-directed mutagenesis and expression in Bordetella sp. for use in recombinant vaccine construction 1995

5/6/8 0153450 DBA Accession No.: 93-11502
Site-specific alterations in the B oligomer that affect receptor-binding activities and mitogenicity of pertussis toxin - Bordetella pertussis toxin recombinant vaccine construction (conference abstract) 1993

5/6/9 0146359 DBA Accession No.: 93-04411
Inactivation of pertussis toxin by site-directed mutation of cell-binding subunits - Bordetella pertussis toxin operon site-directed mutagenesis for potential recombinant vaccine construction (conference abstract) 1993

5/6/10 0143403 DBA Accession No.: 93-01455
New recombinant B oligomer of pertussis toxin - gene cloning and expression in Escherichia coli inclusion body, followed by subunit S2-5 reconstitution and protein renaturation for use in a recombinant vaccine 1992

5/6/11 0134035 DBA Accession No.: 92-06527
The cyclodextrins and their application in environmental biotechnology - cyclodextrin application in e.g. steroid transformation, culture medium for improved pertussis toxin production, mammal cell culture medium serum substitute, etc. (conference paper) 1991

5/6/12 0130700 DBA Accession No.: 92-03192
New acellular anti-pertussis vaccine - pertussis toxin protein engineering; filamentous hemagglutinin and 69 kDa protein production by Bordetella pertussis deletion mutant 1991

5/6/13 0130699 DBA Accession No.: 92-03191
New vaccine molecules obtained by site-directed mutagenesis of the active site of pertussis and diphtheria toxins - Bordetella pertussis recombinant vaccine production (conference paper) 1990

5/6/14 0127026 DBA Accession No.: 91-14668
Site-specific mutagenesis of the catalytic subunit of cholera toxin: substituting lysine for arginine 7 causes loss of activity - potential application as recombinant vaccine component 1991

5/6/15 0125212 DBA Accession No.: 91-12854
Genetically engineered pertussis toxin suitable for vaccine development - site-directed mutagenesis of Bordetella pertussis toxin gene (conference abstract) 1991

5/6/16 0120225 DBA Accession No.: 91-07867
High level heterologous expression in E. coli using mutant forms of the lac promoter - effect of lac promoter point mutation on expression of Bordetella pertussis P69 surface antigen and tetanus toxic fragment C in Escherichia coli 1991

5/6/17 0119754 DBA Accession No.: 91-07396
Detoxification of pertussis toxin by site-directed mutagenesis: a review of Connaught strategy to develop a recombinant pertussis vaccine - Bordetella pertussis TOX operon gene cloning (conference paper) 1991

5/6/18 0116719 DBA Accession No.: 91-04361
Genetic detoxification of pertussis toxin and development of a new vaccine against pertussis - recombinant vaccine construction by site-directed mutagenesis of Bordetella pertussis subunit S1 gene; protein engineering 1990

5/6/19 0116306 DBA Accession No.: 91-03948
Glyphosate tolerant synthase production - glyphosate herbicide resistance 3-enolpyruvoylshikimate-5-phosphate-synthase gene cloning and expression in transgenic plant; enzyme engineering; DNA sequence 1991

5/6/20 0114354 DBA Accession No.: 91-01996
Pertussis toxin mutants and Bordetella strains producing the toxin - having reduced toxicity, use in cellular and acellular vaccine production; Bordetella pertussis, Bordetella parapertussis 1990

5/6/21 0100738 DBA Accession No.: 90-03429
The growth of hybridoma cells in a serum- and protein-free medium - monoclonal antibody production in protein-free, serum-free culture medium (conference paper) 1989

5/6/22 0100477 DBA Accession No.: 90-03168

Engineering bacterial toxins for the development of a new vaccine against whooping cough - Bordetella pertussis toxin gene cloning and expression; protein engineering by site-directed mutagenesis; recombinant vaccine construction (conference paper) 1989

5/6/23 0097524 DBA Accession No.: 90-00215
Recombinant pertussis toxin: enzymatic inactivation of the S1 subunit by a site-specific mutation that conserves its protective epitope - potential recombinant vaccine production (conference abstract) 1988

5/6/24 0093324 DBA Accession No.: 89-11315
Modified pertussis toxin polypeptides - mutagenesis of S1 subunit gene, and expression in Escherichia coli for use in anti-pertussis vaccine 1989

5/6/25 0093321 DBA Accession No.: 89-11312
Immunoprotective, genetically-detoxified pertussis toxin and vaccine - with amino acid substitution(s) or deletion(s) produced by site-directed mutagenesis of toxin gene 1989

5/6/26 0093310 DBA Accession No.: 89-11301
Expression and secretion of the S-1 subunit and C180 peptide of pertussis toxin in Escherichia coli - Bordetella pertussis gene cloning for potential use as recombinant vaccine 1989

5/6/27 0088882 DBA Accession No.: 89-06873
Inactivation of pertussis toxin by site-directed mutagenesis - toxin secretion from Bordetella host, potential application as vaccine (conference abstract) 1989

5/6/28 0088304 DBA Accession No.: 89-06295
Alkylation of cysteine-41, but not cysteine-200, decreases the ADP-ribosyltransferase activity of the S1-subunit of pertussis toxin - Bordetella pertussis gene cloning and expression in Escherichia coli; site-directed mutagenesis and protein engineering of vaccine 1989

5/6/29 0082276 DBA Accession No.: 89-00267
Subunit S1 of pertussis toxin: mapping of the regions essential for ADP-ribosyltransferase activity - potential pertussis vaccine production in recombinant Escherichia coli 1988

5/6/30 0081567 DBA Accession No.: 88-12416
Pertussis toxin S1 mutant with reduced enzyme activity and a conserved protective epitope - potential application as whooping cough vaccine 1988

5/6/31 0075146 DBA Accession No.: 88-05995
Affinity-based chromatography utilizing genetically engineered proteins: interaction of Bordetella pertussis adenylate-cyclase with calmodulin - calmodulin immobilization using a CNBr support 1988

5/6/32 0023848 DBA Accession No.: 84-07123
Antitumor lipopolysaccharide immobilized on insoluble carrier - to reduce toxicity in blood treatment 1984

6/6/1 0175783 DBA Accession No.: 95-02604
DNA encoding Bordetella sp. toxin subunit analogs - recombinant exotoxin subunit S1, S2, S3, S4, S5 analog production by protein engineering for use in pertussis recombinant vaccine 1994

6/6/2 0168379 DBA Accession No.: 94-10930
Construction and characterization of recombinant Vibrio cholerae strains producing inactive cholera toxin analogs - for potential use in vaccine production 1994

6/6/3 0153447 DBA Accession No.: 93-11499
New insights into recombinant pertussis toxin - Bordetella recombinant B oligomer expression in Escherichia coli for potential pertussis recombinant vaccine construction (conference paper) 1993

6/6/4 0135998 DBA Accession No.: 92-08490
Properties of pertussis toxin B oligomer assembled in vitro from recombinant polypeptides produced by Escherichia coli - pertussis toxin subunit purification from inclusion body and assembly into multimeric form for potential development of an acellular recombinant vaccine 1992

6/6/5 0127026 DBA Accession No.: 91-14668
Site-specific mutagenesis of the catalytic subunit of cholera toxin: substituting lysine for arginine 7 causes loss of activity - potential application as recombinant vaccine component 1991

6/6/6 0123956 DBA Accession No.: 91-11598
Protective activity of a recombinant B oligomer of pertussis toxin - expression in Escherichia coli; potential recombinant vaccine (conference abstract) 1991

6/6/7 0113704 DBA Accession No.: 91-01346
The advent of recombinant pertussis vaccines - Bordetella pertussis vaccine construction; pertussis toxin gene cloning and site-directed mutagenesis; recent advances 1990

6/6/8 0099889 DBA Accession No.: 90-02580
Pertussis holotoxoid formed in vitro with a genetically deactivated S1 subunit - application to pertussis recombinant vaccine production 1989

6/6/9 0097524 DBA Accession No.: 90-00215
Recombinant pertussis toxin: enzymatic inactivation of the S1 subunit by a site-specific mutation that conserves its protective epitope - potential recombinant vaccine production (conference abstract) 1988

6/6/10 0097523 DBA Accession No.: 90-00214
Recombinant pertussis toxoid: in vitro formation of holotoxin from recombinant S1 subunits and natural B oligomer - Bordetella pertussis recombinant toxoid, potential application as vaccine (conference abstract) 1988

6/6/11 0088304 DBA Accession No.: 89-06295
Alkylation of cysteine-41, but not cysteine-200, decreases the ADP-ribosyltransferase activity of the S1-subunit of pertussis toxin - Bordetella pertussis gene cloning and expression in Escherichia coli; site-directed mutagenesis and protein engineering of vaccine 1989

6/6/12 0082245 DBA Accession No.: 89-00236
Toward development of a recombinant pertussis vaccine - by expression of Bordetella pertussis toxin site-specific S1 mutant in Escherichia coli (conference abstract) 1988

6/6/13 0081567 DBA Accession No.: 88-12416
Pertussis toxin S1 mutant with reduced enzyme activity and a conserved protective epitope - potential application as whooping cough vaccine 1988

6/6/14 0079321 DBA Accession No.: 88-10170
Identification of a region in the S1 subunit of pertussis toxin that is required for enzymatic activity and that contributes to the formation of a neutralizing antigenic determinant - construction of recombinant Bordetella pertussis toxin plasmids 1988

6/6/15 0078049 DBA Accession No.: 88-08898
Direct expression of Bordetella pertussis toxin subunits to high levels in Escherichia coli - potential whooping cough vaccine preparation 1988

6/6/16 0042275 DBA Accession No.: 86-00123
Hepatitis B vaccines prepared from yeast by recombinant DNA techniques: memorandum from a WHO meeting - surface antigen gene cloning expression and vaccine production in Saccharomyces cerevisiae 1985

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0099889 DBA Accession No.: 90-02580
Pertussis holotoxoid formed in vitro with a genetically deactivated S1 subunit - application to pertussis recombinant vaccine production
AUTHOR: Bartley T D; Whiteley D W; Mar V L; Burns D L; +Burnette W N
CORPORATE AFFILIATE: Amgen CORPORATE SOURCE: Amgen Inc., Thousand Oaks, CA 91320, USA.
JOURNAL: Proc.Natl.Acad.Sci.U.S.A. (86, 21, 8353-57) 1989 CODEN: PNASA6 LANGUAGE: English
ABSTRACT: The cytotoxicity of pertussis toxin produced by Bordetella pertussis is due to the NAD+ ADP-ribosyltransferase (EC-2.4.2.30) activity of the S1 subunit. The recombinant expression of each of the 5 pertussis toxin subunits in Escherichia coli and the production of an enzymatically deficient form of the S1 subunit by site-directed mutagenesis are described. The in vitro assembly of holotoxin from native pertussis toxin B oligomer and recombinant S1 subunits is discussed. Holotoxin assembled with recombinant S1 of authentic

amino acid sequence was indistinguishable from native pertussis toxin in its electrophoretic migration and ability to elicit a cytopathic response in cultured Chinese hamster ovary cells; holotoxin assembled with the genetically deactivated analog of recombinant S1 displayed greatly diminished cytopathicity. These results verify that the in vitro cytopathic effects of pertussis toxin are the result of the enzymatic activity of the S1 subunit. The formation of pertussis holotoxin from an enzymatically deficient analog subunit may result in a new and safer pertussis toxoid vaccine. (46 ref)

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0079321 DBA Accession No.: 88-10170
Identification of a region in the S1 subunit of pertussis toxin that is required for enzymatic activity and that contributes to the formation of a neutralizing antigenic determinant - construction of recombinant Bordetella pertussis toxin plasmids
AUTHOR: Cieplak W; Burnette W N; Mar V L; Kaljot K T; Morris C F; +Keith J M
CORPORATE AFFILIATE: AMGen
CORPORATE SOURCE: National Institute of Allergy and Infectious Diseases, Laboratory of Pathobiology, Rocky Mountain Laboratories, Hamilton, MT 59840, USA.
JOURNAL: Proc.Natl.Acad.Sci.U.S.A. (85, 13, 4667-71) 1988 CODEN: PNASA6 LANGUAGE: English
ABSTRACT: Pertussis toxin (PTX) is produced by Bordetella pertussis and may have a central role in the pathogenesis of whooping cough. The enzymatic activity of PTX is associated with the S1 subunit and 2 regions of the S1 subunit possess sequence homology with the A fragments of cholera toxin and Escherichia coli heat-labile toxin. Plasmid pPTXS1/2, containing the phage lambda PL promoter, and the DNA sequence encoding the S1 subunit minus the leader sequence, was constructed. Plasmid pPTXS1/1 was opened at the initiation codon and treated with BAL-31 exonuclease. At various times, aliquots were removed and incubated at 65 deg to stop the reaction. The region carrying the PL promoter and the ribosome binding site was isolated from the parent vector plasmid pCFM4722 and ligated with the BAL-31 generated 3-3.5 kb isolates from plasmid pPTXS1/2. E. coli FM5 cells were transformed with the truncated S1 expression plasmids. A series of 6 truncated recombinant S1 proteins was produced at high levels. The region of homology located near the amino terminus of S1 was identified as an apparent enymatic subsite potentially important antigenic determinant. (39 ref)

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0078049 DBA Accession No.: 88-08898
Direct expression of Bordetella pertussis toxin subunits to high levels in Escherichia coli - potential whooping cough vaccine preparation
AUTHOR: Burnette W N; Mar V L; Cieplak W; Morris C F; Kaljot K T; Marchitto K S
CORPORATE AFFILIATE: AMGen
CORPORATE SOURCE: Amgen Inc., 1900 Oak Terrace Lane, Thousand Oaks, CA 91320, USA.
JOURNAL: Bio/Technology (6, 6, 699-706) 1988 CODEN: BTCHDA LANGUAGE: English
ABSTRACT: The individual Bordetella pertussis toxin subunit gene segments were isolated with or without their signal peptides and cloned into the Escherichia coli generalized cloning vector plasmid pCFM, which possesses the large phage lambda promoter, an E. coli ribosome binding site and a cloning cluster. The vector was used to transform E. coli FM5, a K12 derivative. The S1 subunit was synthesized at over 10% of the total cell protein with its natural leader peptide, and at a much reduced level as a mature methionyl polypeptide. Recombinant S1 preparations possessed NAD-ase (EC-3.2.2.5) and ADP-ribose-5-phosphate- adenyltransferase (EC-2.7.7.35) activities identical to those of native pertussis toxin. Subunits S2, S3, S4 and S5 were also individually expressed in E. coli, both as mature methionyl proteins and with their authetic signal sequences. The ability to obtain significant amounts of non-fusion recombinant subunits will facilitate the identification of functional and enzymatic sites within the toxin, the mapping of neutralizing and protective antigenic epitopes, and will allow their assessment as subunit whooping cough vaccines. (66 ref)

7/6/1 0127026 DBA Accession No.: 91-14668
Site-specific mutagenesis of the catalytic subunit of cholera toxin: substituting lysine for arginine 7 causes loss of activity - potential application as recombinant vaccine component 1991

7/6/2 0097524 DBA Accession No.: 90-00215
Recombinant pertussis toxin: enzymatic inactivation of the S1 subunit by a site-specific mutation that conserves its protective epitope - potential recombinant vaccine production (conference abstract) 1988

7/6/3 0088304 DBA Accession No.: 89-06295
Alkylation of cysteine-41, but not cysteine-200, decreases the ADP-ribosyltransferase activity of the S1-subunit of pertussis toxin - Bordetella pertussis gene cloning and expression in Escherichia coli; site-directed mutagenesis and protein engineering of vaccine 1989

7/6/4 0081567 DBA Accession No.: 88-12416
Pertussis toxin S1 mutant with reduced enzyme activity and a conserved protective epitope - potential application as whooping cough vaccine 1988

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0081567 DBA Accession No.: 88-12416
Pertussis toxin S1 mutant with reduced enzyme activity and a conserved protective epitope - potential application as whooping cough vaccine
AUTHOR: Burnette W N; Cieplak W; Mar V L; Kaljot K T; Sato H; Keith J M
CORPORATE AFFILIATE: AMGen CORPORATE SOURCE: Amgen, Thousand Oaks, CA 91320, USA.
JOURNAL: Science (242, 4875, 72-74) 1988 CODEN: SCIEAS LANGUAGE: English
ABSTRACT: Pertussis factor (PTX) is a major virulence factor in whooping cough and can elicit protective antibodies. Amino acid residues 8 to 15 of PTX subunit S1 are important for the ADP-ribosyltransferase (EC-2.4.2.30) activity associated with the pathobiological effects of PTX. This region contains at least a portion of a epitope that elicits both toxin-neutralizing and protective antibody responses in mice. Oligonucleotides were synthesized that incorporated a series of substitution mutations. A mutation was also designed that allowed for selective deletion of the homology region. The gene encoding the S1 subunit was subjected to site-specific mutagenesis, and the phenotypic effects were examined by assaying the mutant S1 polypeptides for their ability to react with the protective monoclonal antibody 1 B7. A mutant containing a single amino acid substitution (arg to lys) had reduced enzymatic activity (0.02% of control) while retaining the protective epitope. This analog S1 molecule may provide the basis for a genetically detoxified PTX with potential for use as a component of an acellular vaccine against whooping cough. (27 ref)